

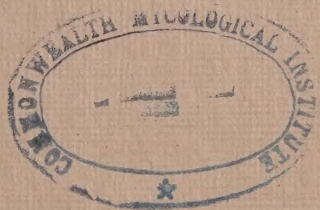
Studies on the Development of Butter Cultures from Mixtures of Organisms

BY R. S. FARMER AND B. W. HAMMER

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
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DAIRY INDUSTRY SECTION



RESEARCH BULLETIN NO. 146
DECEMBER, 1931
AMES, IOWA

December, 1931

Research Bulletin No. 146

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Studies on the Development of Butter Cultures from Mixtures of Organisms

BY R. S. FARMER AND B. W. HAMMER

Studies on the bacteriology of butter cultures have shown that two types of bacteria are normally present, one which attacks primarily the lactose with the formation of large amounts of lactic acid, while the other is characterized by its production of volatile acid from citric acid. These findings suggest the development of butter cultures under carefully controlled conditions by making mixtures of the two types of organisms. While many satisfactory butter cultures have been secured with this procedure, mixtures made with organisms that appear typical on the basis of morphology, cultural characteristics and the usual biochemical tests, often fail to develop a satisfactory aroma and flavor and, accordingly, the percentage of the attempts that are successful is small.

The work herein reported was carried out to determine some of the factors of importance in the development of butter cultures from mixtures of *Streptococcus lactis* and one of the citric acid fermenting streptococci (*Streptococcus citrovorus* and *Streptococcus paracitrovorus*).

METHODS

GENERAL METHOD OF CARRYING BUTTER CULTURES

The usual method of carrying butter cultures in the laboratory involved the use of 120 ml., glass stoppered bottles. Commercially pasteurized milk was transferred to these bottles and re-pasteurized by standing the bottles in a water bath, raising the temperature of the milk to about 82° C. and holding for about 30 minutes. The milk was cooled to 21° C. by running cold water into the hot water surrounding the bottles. In transferring the butter cultures, sterile glass tubes were used. The incubation temperature was 21° C. and the normal incubation period was about 15 hours. After incubation, the cultures were held in a cooler at about 7° C. until the next transfer was made.

GENERAL METHOD OF COMBINING BUTTER CULTURE ORGANISMS AND PROPAGATING THE MIXTURES

The general method of combining a citric acid fermenting *Streptococcus* and *S. lactis* for the purpose of developing a butter culture was as follows: The citric acid fermenter was inoculated

into a test tube of litmus milk in the evening and allowed to grow at about 21° C. overnight; *S. lactis* was then added. When the milk containing the mixture of organisms was coagulated, it was added to milk that had been pasteurized by the method regularly used in the handling of butter cultures. The pasteurized milk culture was incubated at 21° C. until coagulated and then held in a cooler at about 7° C. until the regular butter culture transfers were made.

METHODS FOR THE ISOLATION AND IDENTIFICATION OF THE *S. LACTIS* STRAINS

The method employed for the isolation of the *S. lactis* strains consisted of plating raw, sweet or sour cream on whey agar and incubating the plates at 21° C. As soon as colonies suggesting streptococci were well developed, some of them were picked into tubes of litmus milk and the milk incubated at 21° C. Cultures coagulating litmus milk within three days at 21° C., with reduction of the litmus but without digestion or the formation of gas, and which appeared in stained preparations from milk as a gram positive coccus arranged in chains or pairs were considered to be *S. lactis*. In the differentiation into varieties, the classification of Hammer and Baker¹ was followed.

METHODS FOR THE ISOLATION AND IDENTIFICATION OF THE CITRIC ACID FERMENTING STREPTOCOCCI

The methods employed for the isolation of the citric acid fermenting streptococci were as follows:

From Cream. Samples of raw cream were incubated at 21° C. for about four days and then plated on whey agar. After incubating the plates for from two to five days at 21° C., 20 to 30 colonies that had the appearance of Streptococcus colonies were picked into litmus milk. A large percentage of the cultures coagulated the milk rapidly and were *S. lactis* types. Cultures curdling milk slowly, or not at all, yet showing a slight reddening of the litmus or a slight reduction at the bottom of the tube and appearing in stained preparations from milk as a gram positive coccus arranged in pairs or in chains, were surmised to be citric acid fermenting streptococci.²

From Butter. Samples of butter secured with a sterile trier were inoculated into tubes of warm (about 45° C.) litmus milk, the milk shaken and then incubated at about 21° C. for about three days; organisms surmised to be citric acid fermenting

¹Hammer, B. W., and Baker, M. P. Classification of the *Streptococcus lactis* group. Iowa Agr. Exp. Sta., Res. Bul. 99. 1926.

²Hammer, B. W. Volatile acid production of *S. lactis* and the organisms associated with it in starters. Iowa Agr. Exp. Sta., Res. Bul. 63. 1920.

Hammer, B. W., and Baker, M. P. Studies on *Streptococcus paracitrovorus* group. Iowa Agr. Exp. Sta., Res. Bul. 81. 1923.

streptococci were secured from the milk by the procedure used with cream.

After purifying by plating, a culture that was to be examined for its action on citric acid was inoculated into two flasks, each containing 325 ml. of sterile skim milk. Citric acid was added to one of the flasks; this was done by agitating the contents with a rotary motion and then pouring in a sterile solution of 1.3 grams (0.4 percent of 325 grams) of citric acid crystals in 10 ml. of distilled water. Following incubation at 21° C. for seven days, the volatile acidities produced by the organism were determined.

Two methods of determining volatile acidity were used. The standard method was the one employed by Hammer and Bailey³ and consisted of distilling a 250-gram portion of fermented milk with steam, after the addition of 15 ml. of approximately N/1 H₂SO₄, until 1,000 ml. of distillate were secured; the distillate was titrated with N/10 NaOH, using phenolphthalein as an indicator, and the results expressed as the milliliters of N/10 NaOH required.

Preliminary results were often secured on the milk to which citric acid had been added by a micro procedure, a 50-gram portion of the milk being distilled with steam, after the addition of 3 ml. of approximately N/1 H₂SO₄, until 10 ml. of distillate were secured and then titrating the distillate with N/20 NaOH, using phenolphthalein as an indicator; if 0.9 ml. or more of N/20 NaOH was required, the volatile acidities produced by the organism in milk and in milk plus citric acid were determined with the standard method. The micro method detected satisfactorily the cultures which produced very little volatile acid and thus made it possible to eliminate rapidly the organisms which were of no interest from the standpoint of the development of butter cultures.

When the volatile acidity produced in milk was definitely increased by the addition of citric acid, the organism was considered to be a citric acid fermenter.

EXPERIMENTAL

I. INFLUENCE OF VARIOUS STRAINS OF THE CONSTITUENT ORGANISMS IN THE DEVELOPMENT OF BUTTER CULTURES

INFLUENCE OF VARIOUS STRAINS OF *S. LACTIS*

The variations that occur in the organisms of the *S. lactis* group suggest that some of them would be much more satisfactory than others for the development of butter cultures. On the basis of the variations readily detectable in milk, Hammer

³Hammer, B. W., and Bailey, D. E. The volatile acid production of starters and of organisms isolated from them. Iowa Agr. Exp. Sta., Res. Bul. 55. 1919.

and Baker⁴ have subdivided and classified the species and varieties of the *S. lactis* group as follows: *S. lactis* var. *maltigenes*, *S. lactis* var. *hollandicus*, *S. lactis* var. *anoxyphilus*, *S. lactis* var. *tardus*, *S. thermophilus* and typical *S. lactis* which shows none of the unusual characters shown by the other types.

A number of the *S. lactis* varieties have characters which definitely make them undesirable in butter cultures. The malty odor and flavor of *S. lactis* var. *maltigenes* is very objectionable in all dairy products, and butter cultures showing maltiness would decrease rather than increase the quality of butter. The ropiness produced by *S. lactis* var. *hollandicus* does not influence the aroma and flavor of butter cultures but the condition is undesirable from the standpoint of handling both butter cultures and cream. The slow coagulation that occurs with *S. lactis* var. *tardus* excludes this variety from use in the development of butter cultures; in practice, butter cultures must coagulate milk in a reasonable period because equipment should be used efficiently and also because extended incubation periods favor the growth of contaminating organisms. The high temperature required for the rapid growth of *S. thermophilus* is definitely objectionable in the carrying of butter cultures because it entirely prevents, or seriously limits, the growth of the citric acid fermenters and, moreover, it greatly favors the development of organisms resisting the pasteurization exposures used with the milk.

The only one of the objectionable *S. lactis* varieties studied in connection with the development of butter cultures was *S. lactis* var. *maltigenes*. Cultures of this variety were frequently encountered among those secured when colonies were picked into litmus milk from whey agar plates poured with sour milk or cream, and a number of them were used in combinations with citric acid fermenting streptococci. Variations were noted in the extent of the maltiness produced by the different strains of *S. lactis* var. *maltigenes*, but in all cases the objectionable malty odor and flavor persisted down through a series of transfers of the mixtures.

A number of strains of *S. lactis* var. *anoxyphilus* were secured by making frequent observations for slow reduction on the cultures picked into litmus milk from whey agar plates poured with sour milk or cream. In combinations with cultures of citric acid fermenting streptococci the *S. lactis* var. *anoxyphilus* strains gave a number of fairly satisfactory butter cultures, and the combinations coagulated at about the rate desired in a butter culture. The *S. lactis* var. *anoxyphilus* strains that were studied showed variations in the rates of reduction,

⁴Hammer, B. W., and Baker, M. P. Classification of the *Streptococcus lactis* group. Iowa Agr. Exp. Sta., Res. Bul. 99, 1926.

but there was no correlation between these rates and the quality of the butter cultures.

Many typical *S. lactis* strains were studied in connection with the development of butter cultures. These were secured from the cultures that developed when colonies were picked into litmus milk from whey agar plates poured with various dairy products. The odors and flavors of these cultures, as judged from transfers made to milk pasteurized with a comparatively high exposure, were extremely variable; some were conspicuously sour and high-acid, some had a flavor suggesting cabbage and some had a mild, clean flavor. Six *S. lactis* cultures showing pronounced variations in flavor were selected and each was combined with each of six citric acid fermenting streptococci and also carried alone in pasteurized milk. A culture which produced a sharp, high-acid flavor when grown alone gave this same condition in combinations with citric acid fermenters through a series of transfers, and it appeared that the citric acid fermenters generally had little influence on the odor and flavor produced by it; presumably the rapid growth of the *S. lactis* strain made conditions unfavorable for the citric acid fermenters used. Two cultures which produced a flavor suggesting cabbage when grown alone produced this same condition in combinations with certain citric acid fermenters, but with others satisfactory butter cultures were secured. The three *S. lactis* strains which produced a clean, mild flavor when grown alone sometimes yielded satisfactory butter cultures when combined with citric acid fermenters and sometimes did not. It appeared that many combinations were unsatisfactory, even when the *S. lactis* strain produced no objectionable odor and flavor, and that a *S. lactis* strain which yielded a good butter culture with one of the citric acid fermenters might give unsatisfactory results with another. When satisfactory results were secured the striking influence of the citric acid fermenter was evident when the aroma and flavor of the combination was compared with the aroma and flavor of the *S. lactis* strain, and such a comparison also showed how greatly a butter culture differs from a pure culture of *S. lactis*, even when the latter produces a clean, mild odor and flavor.

An additional series of combinations was made using typical *S. lactis* strains, which produced no definitely objectionable condition and still were not clean and mild in flavor, and various citric acid fermenters; some satisfactory butter cultures were secured while other mixtures had odors and flavors essentially like those of the *S. lactis* strain used.

In general, the results secured indicated that *S. lactis* strains having no objectionable odor and flavor gave satisfactory butter cultures in combination with certain citric acid fermenters while with others they did not. Apparently only certain combinations

were satisfactory and *S. lactis* strains which developed satisfactorily with one citric acid fermenter might fail with another. It should be noted that the strains of *S. lactis* and citric acid fermenters used were unselected.

S. lactis strains were isolated from eight butter cultures secured from various commercial laboratories. Six of the butter cultures were coarse and high-acid in flavor and the *S. lactis* strains secured from these produced a sharp, high-acid flavor in milk. The other two butter cultures had a mild, clean flavor and yielded *S. lactis* strains that produced a mild, clean flavor. When *S. lactis* strains from the eight sources were combined with citric acid fermenters, the odors and flavors of the *S. lactis* strains were sometimes influenced by the citric acid fermenters and sometimes not; if there was such an influence the combinations made with the sharp, high-acid *S. lactis* strains were coarse, while those made with the less acid *S. lactis* strains were satisfactory.

INFLUENCE OF VARIOUS STRAINS OF CITRIC ACID FERMENTERS

Seventy-one citric acid fermenting streptococci were isolated and studied for their volatile acid production and ability to produce satisfactory butter cultures when combined with *S. lactis* A, a strain that had been found very useful in the development of butter cultures. The results obtained, together with the sources of the organisms, are given in table I.

The data show that the volatile acidities produced in milk varied from 7.1 to 30.0 and averaged 20.3 while in milk plus 0.4 percent citric acid the volatile acidities ranged from 25.0 to 84.8 and averaged 57.2. The strains from the two main sources, sour cream and imported butter, showed no conspicuous differences in the volatile acidities produced. In combination with *S. lactis* A, 10 of the strains gave fair, fair to good, or good butter cultures, while with the other 61 the desired aroma and flavor were lacking. All of the eight citric acid fermenters isolated from imported butter failed to give a satisfactory butter culture in combination with *S. lactis* A. There was no correlation between the amount of volatile acidity produced and the ability of a strain to develop a satisfactory butter culture in combination with *S. lactis* A.

Each of 12 citric acid fermenters (Cultures 1 to 12 inc., table I) was combined with each of 6 *S. lactis* strains (including *S. lactis* A) that varied considerably in the odor and flavor produced when grown in milk and the combinations carried through a series of transfers. The combinations containing cultures 1, 4 and 8 were commonly more satisfactory than those containing the other citric acid fermenters and culture 1 rather regularly gave the most desirable combinations. The mixtures in which *S. lactis* A was used were usually better than those in which the other *S. lactis* strains were employed; the *S. lactis* strains pro-

TABLE I. SOURCES, VOLATILE ACIDITIES AND VALUES IN THE DEVELOPMENT OF BUTTER CULTURES WHEN COMBINED WITH *S. LACTIS*
A OF 71 CITRIC ACID FERMENTING STREPTOCOCCI

Culture number	Source	Volatile acid production* in		Aroma and flavor of butter culture obtained in combination with <i>S. lactis</i> A
		Milk	Milk plus 0.4% citric acid	
1	Butter culture	23.5	63.5	Good
2	Sour cream	23.0	63.2	Lacking
3	Sour cream	26.6	52.0	Lacking
4	Sour cream	26.8	54.3	Fair
5	Sour cream	23.5	48.6	Lacking
6	Sour cream	21.5	45.9	Lacking
7	Sour cream	15.2	25.0	Lacking
8	Sour cream	12.0	65.0	Fair
9	Sour cream	20.0	57.0	Lacking
10	Sour cream	12.5	35.8	Lacking
11	Sour cream	7.1	62.0	Lacking
12	Sour cream	8.0	49.0	Lacking
13	Sour cream	24.5	53.5	Lacking
14	Sour cream	17.0	57.5	Fair
15	Sour cream	29.5	62.5	Good
16	Sour cream	27.8	75.1	Lacking
17	Sour cream	11.2	46.8	Lacking
18	Sour cream	24.4	49.5	Lacking
19	Sour cream	11.2	48.0	Lacking
20	Sour cream	17.2	46.2	Lacking
21	Sour cream	19.6	60.0	Lacking
22	Sour cream	25.1	70.8	Good
23	Sour cream	23.4	45.0	Lacking
24	Sour cream	28.0	57.5	Good
25	Sour cream	19.5	47.0	Good
26	Sour cream	28.0	54.2	Lacking
27	Sour cream	10.5	53.1	Lacking
28	Sour cream	19.5	60.8	Lacking
29	Sour cream	20.3	49.6	Lacking
30	Sour cream	15.0	43.2	Fair to good
31	Sour cream	16.5	50.0	Fair to good
32	Sour cream	21.2	57.5	Lacking
33	Sour cream	16.0	61.0	Lacking
34	Sour cream	18.6	62.2	Lacking
35	Sour cream	20.4	47.0	Lacking
36	Sour cream	18.2	55.2	Lacking
37	Sour cream	17.0	61.3	Lacking
38	Sour cream	20.0	75.0	Lacking
39	Sour cream	19.0	77.5	Lacking
40	Sour cream	22.0	65.0	Lacking
41	Sour cream	20.5	65.0	Lacking
42	Sour cream	22.3	57.0	Lacking
43	Sour cream	15.7	57.6	Lacking
44	Sour cream	17.3	62.9	Lacking
45	Sour cream	18.4	46.3	Lacking
46	Sour cream	20.0	60.1	Lacking
47	Sour cream	19.0	46.8	Lacking
48	Sour cream	26.0	69.0	Lacking
49	Sour cream	25.9	65.5	Lacking
50	Sour cream	19.9	44.5	Lacking
51	Sour cream	19.0	74.4	Lacking
52	Sour cream	22.2	68.7	Lacking
53	Sour cream	12.0	58.6	Lacking
54	Sour cream	21.2	49.8	Lacking
55	Sour cream	23.3	72.0	Lacking
56	Sour cream	22.1	48.1	Lacking
57	Sour cream	8.5	76.0	Lacking
58	Sour cream	26.0	39.6	Lacking
59	Sour cream	23.5	52.5	Lacking
60	Sour cream	23.9	39.1	Lacking
61	Sour cream	30.0	78.5	Lacking
62	Sour cream	22.5	65.5	Lacking
63	Sour cream	27.4	84.8	Lacking
64	Danish butter	22.3	66.5	Lacking
65	Danish butter	21.9	60.0	Lacking
66	Irish butter	20.6	67.9	Lacking
67	Irish butter	21.6	71.3	Lacking
68	Irish butter	23.4	74.2	Lacking
69	Siberian butter	22.4	40.8	Lacking
70	Siberian butter	22.0	37.8	Lacking
71	Siberian butter	18.0	46.4	Lacking

*The values given represent the ml. of N 10 NaOH required for the neutralization of the first 1,000 ml. of distillate obtained when a 250-gram portion of a 7-day old culture was distilled with steam after the addition of 15 ml. of approximately N/1 H₂SO₄.

ducing considerable undesirable flavor when grown alone were regularly less satisfactory than those with a clean, mild flavor. With some of the combinations the flavor was as lacking as with a *S. lactis* culture.

The citric acid fermenters varied considerably in the odors produced in pure cultures in milk, some giving an odor definitely suggesting a butter culture while others did not; when a butter culture aroma was produced it was evident only after an incubation of several days. Citric acid fermenters that produced a butter culture aroma sometimes developed a good butter culture when combined with a *S. lactis* strain having a clean, mild flavor and sometimes did not and the same was true of cultures that did not produce a butter culture aroma. The strains that gave the most pronounced butter culture aroma were those producing considerable total acid. Presumably, with a higher total acid a smaller percentage of the volatile acids was held in combinations with the milk constituents.

In order to determine whether or not the rate of volatile acid production of a citric acid fermenter was of importance in the development of a butter culture, each of six citric acid fermenters, three (cultures 1, 24 and 25) which yielded satisfactory butter culture when combined with *S. lactis* A, and three (cultures 2, 26 and 29) which did not, was inoculated into seven flasks of sterile milk, the milk incubated at about 21° C. and volatile acid determinations made after 1, 2, 3, 5, 7, 9 and 12 days. The results obtained are given in table II.

TABLE II. COMPARATIVE VOLATILE ACID PRODUCTION OF CITRIC ACID FERMENTING STREPTOCOCCI YIELDING SATISFACTORY AND UNSATISFACTORY BUTTER CULTURES

Incubation	Volatile acid production* of citric acid fermenting streptococci yielding					
	Satisfactory butter cultures			Unsatisfactory butter cultures		
	Culture 1	Culture 24	Culture 25	Culture 2	Culture 26	Culture 29
1 day	6.8	2.1	1.2	3.3	2.2	1.5
2 days	14.4	8.3	8.5	9.6	7.1	4.5
3 days	23.5	11.8	12.5	12.2	10.9	9.3
5 days	23.6	19.7	19.2	17.0	19.4	20.0
7 days	23.0	21.4	24.5	22.8	25.2	22.3
9 days	23.3	28.4	25.7	23.3	30.7	24.7
12 days	25.3	27.9	25.3	25.5	29.8	26.8

*See table I.

The data show that the volatile acid production of cultures 1 and 2 was essentially the same after 7, 9 and 12 days, and that culture 1 which invariably yielded more satisfactory butter cultures than culture 2, produced more volatile acid during a short incubation period. Cultures 24, 25, 26 and 29, however, did not show conspicuous variations in the rates of volatile acid production (although culture 29 produced comparatively little volatile

acid after two days) and two of them yielded satisfactory butter cultures while two did not.

GENERAL OBSERVATIONS ON THE DEVELOPMENT OF BUTTER CULTURES BY COMBINING ORGANISMS

It was evident from the trials carried out that a certain combination of a citric acid fermenter and a *S. lactis* strain was very constant in its ability or inability to develop a satisfactory butter culture. If a satisfactory butter culture was secured the first time the organisms were combined, a comparable result was regularly secured in later attempts, while if the first combination was unsatisfactory further trials were also unsuccessful.

The rapidity with which butter cultures could be developed varied with different combinations. In some instances a butter culture aroma and flavor would be evident in the first transfer in pasteurized milk, while in other instances several transfers would be lacking in aroma and flavor and the desired aroma and flavor then appear. Certain combinations produced some butter culture aroma and flavor in the first few transfers while later transfers lacked the desired aroma and flavor.

II. RELATIONSHIP OF ORGANISMS IN SATISFACTORY MIXTURES OF BUTTER CULTURE ORGANISMS

In the series of trials in which each of 6 *S. lactis* strains was combined with each of 12 citric acid fermenters, one of the most satisfactory combinations was *S. lactis* A with citric acid fermenter 1. Many additional *S. lactis* strains were combined with citric acid fermenter 1 and many additional citric acid fermenters with *S. lactis* A. The strains yielding satisfactory butter cultures (the *S. lactis* strains in combination with citric acid fermenter 1 and the citric acid fermenters in combination with *S. lactis* A) were then employed in various combinations, and it was found that each *S. lactis* strain yielded a satisfactory butter culture in combination with each citric acid fermenter. These results indicated that (a) a *S. lactis* strain selected on the basis of its ability to produce a satisfactory butter culture when combined with the citric acid fermenter of a certain combination would also combine satisfactorily with the citric acid fermenters that produced satisfactory butter cultures in mixtures with the *S. lactis* of the combination and (b) that a citric acid fermenter selected on the basis of its ability to produce a satisfactory butter culture when combined with the *S. lactis* of a certain combination would also combine satisfactorily with the *S. lactis* strains that produced satisfactory butter cultures in mixtures with the citric acid fermenters of the combination.

It should be emphasized that when the *S. lactis* and citric acid fermenters were unselected, a *S. lactis* strain that combined satisfactorily with one citric acid fermenter might not combine

satisfactorily with another and also that a citric acid fermenter which combined satisfactorily with one *S. lactis* strain might not combine satisfactorily with another.

III. RAPID PROCEDURES FOR THE SELECTION OF ORGANISMS SATISFACTORY FOR THE DEVELOPMENT OF BUTTER CULTURES

While the value of a particular combination of organisms for the development of butter cultures cannot be foretold from the general characters of the organisms, useful information on each type can be secured by procedures that are easily carried out.

SELECTING A *S. LACTIS* STRAIN

Certain *S. lactis* strains can be recognized as definitely objectionable, from the standpoint of developing butter cultures, by their action in milk. A culture should coagulate milk fairly rapidly and the coagulated milk should be free from ropiness and definitely objectionable odors and flavors, such as high-acid, cabbage, malt, etc. Cultures meeting these requirements can then be combined with a citric acid fermenter that has proved useful in the development of butter cultures and a more careful selection made. The mixtures of the *S. lactis* strains with the citric acid fermenter can be transferred a number of times in tubes of sterile litmus milk and then inoculated into pasteurized milk. A culture of each of the *S. lactis* strains alone is necessary if the exact influence of the citric acid fermenter is to be determined. With this procedure *S. lactis* strains that were very useful in the development of butter cultures have been selected. The pasteurized milk cultures of *S. lactis* gave a much better idea of the odor and flavor produced than the sterile litmus milk cultures, and frequently a culture which seemed to be quite satisfactory in sterile litmus milk was recognized as undesirable when it was grown in pasteurized milk.

SELECTING A CITRIC ACID FERMENTER

An organism identified as a citric acid fermenting Streptococcus can be easily studied from the standpoint of its usefulness for developing butter cultures by mixing it with *S. lactis* strains having mild, clean flavors and making a number of transfers of the combinations. The identification of a large number of citric acid fermenters is time-consuming, however, and seems especially unnecessary since a large percentage of the organisms is unsatisfactory for developing butter cultures.

A procedure has been used for securing citric acid fermenters, suitable for the development of butter cultures, that does not involve the preliminary identification of the organisms. The materials, such as sour cream, from which isolations are to be made

are plated on whey agar, the plates incubated from two to five days at 21° C. and colonies suggesting streptococci then picked into litmus milk. The cultures that appear to be *S. lactis* are discarded, while each of those that shows a change suggesting a citric acid fermenter is combined with *S. lactis* strains. The mixtures are carried through a number of transfers in litmus milk and then put into pasteurized milk. With this method organisms that give satisfactory butter cultures in combination with suitable *S. lactis* strains have often been secured. When studied in detail, including the volatile acid production in milk and in milk plus citric acid, these cultures have all proved to be citric acid fermenting streptococci.

IV. STABILITY OF BUTTER CULTURES

Although all of the transfers of a butter culture carried under rather careful conditions are not equally good, with reasonably uniform methods of propagation butter cultures often show a surprising stability. Various studies that have been carried out illustrate this stability.

INFLUENCE OF THE RELATIVE NUMBER OF EACH TYPE OF ORGANISM IN THE ORIGINAL MIXTURE

The influence of the relative number of each type of organism in the original mixture on the aroma and flavor of a butter culture was studied, using eight citric acid fermenters and a *S. lactis* strain that had been found satisfactory for the development of butter cultures. Various combinations of each of the citric acid fermenters and the *S. lactis* strain were made. Combination 1 was made by adding one loop of a five-day litmus milk culture of the citric acid fermenter to 10 ml. of a recently coagulated culture of the *S. lactis* strain; combination 2 was made by adding one loop of a five-day culture of the citric acid fermenter and 1 ml. of a recently coagulated culture of the *S. lactis* strain to 10 ml. of sterile litmus milk; combination 3 consisted of one loop of a young culture of each organism in 10 ml. of sterile litmus milk; combination 4 was made by adding one loop of a recently coagulated culture of the *S. lactis* strain to 10 ml. of a one-day culture of the citric acid fermenter; and combination 5 was made by adding one loop of a recently coagulated culture of the *S. lactis* strain to 10 ml. of a three-day culture of the citric acid fermenter.

With each citric acid fermenter, combinations 1, 2 and 3 coagulated rapidly, while combinations 4 and 5 coagulated slowly, four days being required with some of the mixtures. Presumably large numbers of the citric acid fermenters tended to inhibit the *S. lactis* strain;⁵ although the procedure used did not permit

⁵Hammer, B. W. Volatile acid production of *S. lacticus* and the organisms associated with it in starters. Iowa Agr. Exp. Sta., Res. Bul. 63. 1920.

careful comparisons of the periods required for the coagulation with the various combinations, it appeared that the restraining action varied considerably with the different citric acid fermenters. Each combination was carried through five transfers in 10 ml. quantities of sterile litmus milk at 21° C., using a loop of inoculating material, and was then put into pasteurized milk. During a number of transfers in pasteurized milk, comparisons were made of the combinations in each series. The five butter cultures secured with each of the citric acid fermenters were essentially the same, and the minor variations that occurred were apparently related to variations in the total acidities. These results indicated that regardless of the comparative numbers of the two types in the original mixture, the organisms established the same relationship so that the same general aroma and flavor development occurred.

INFLUENCE OF ADDING CITRIC ACID FERMENTING STREPTOCOCCI TO BUTTER CULTURES

One of the common defects of butter cultures is a lack of aroma and flavor, which presumably is due to the failure of the citric acid fermenters to develop properly. In a number of instances a butter culture showing this condition was transferred to pasteurized milk and a citric acid fermenter that had been found satisfactory for the development of butter cultures was also added. This new mixture was then compared with the butter culture alone through a series of transfers, using the method ordinarily employed in the carrying of butter cultures. The addition of a citric acid fermenter regularly failed to improve the butter cultures.

TRANSFERRING BUTTER CULTURES IN STERILE LITMUS MILK

Twenty-three butter cultures were carried in sterile litmus milk in test tubes (about 10 ml. per tube), the tubes being incubated at room temperature, which was approximately 21° C., and transferred regularly every other day with a loop. After two weeks, four weeks and eight weeks, coagulated litmus milk cultures were inoculated into pasteurized milk and the aromas and flavors of the resulting cultures compared with those of cultures from the same sources that had been carried in pasteurized milk with the usual procedure.

The cultures coming from the series of transfers in litmus milk at room temperature, using two-day incubation periods, were regularly as satisfactory as those transferred with the usual method, even when the period in litmus milk was as long as eight weeks. These results indicated that butter cultures could be carried satisfactorily in sterile litmus milk and that the over-ripening which followed the long period of incubation was not

harmful. The procedure may be useful when large numbers of butter cultures are to be carried for extended periods.

TRANSFERRING BUTTER CULTURES BEFORE COAGULATION

Fifteen butter cultures were carried through a series of rapid transfers in tubes of litmus milk at about 21° C., the transfers being made so frequently that coagulation had not occurred when the inoculating material was removed from a tube; after 5, 10, 15 and 20 days, uncoagulated litmus milk transfers were inoculated into pasteurized milk and the aromas and flavors of the resulting cultures compared with those of cultures from the same sources that had been carried in pasteurized milk with the usual procedure. The cultures secured after rapid transfers for 5 days were as satisfactory as those with which the usual method of transferring had been employed, while after rapid transfers for 10 days, eight were satisfactory and seven produced less desirable aromas and flavors than the cultures with which the usual method of transferring had been employed. When the seven cultures which were lacking in aroma and flavor were propagated with the usual procedure, they quickly became satisfactory and remained so through a series of transfers. All the cultures secured after rapid transfers for 15 or 20 days had less desirable aromas and flavors than the cultures with which the usual method had been used. When these were propagated in the usual manner, they failed to develop satisfactory aromas and flavors.

The results showed that the two constituent organisms of butter cultures tended to maintain a relationship that was satisfactory for the production of a desirable aroma and flavor even when the milk in which they grew was not coagulated during a series of transfers, but that it was possible with a long series of rapid transfers to destroy the desired relationship between the two constituent organisms.

V. INFLUENCE OF TEMPERATURE ON BUTTER CULTURES

While temperature is one of the important factors influencing the growth of all microorganisms, its effect would be expected to be especially important in connection with butter cultures since, in these, a proper balance between two types of organisms must be maintained if satisfactory results are to be secured.

INFLUENCE OF INCUBATION TEMPERATURE

The influence of the incubation temperature on butter cultures was studied by carrying six cultures through five transfers in pasteurized milk at each of six temperatures—37°, 30°, 25.5°, 21°, 19° and 15° C.—and examining each transfer for aroma and flavor. The final transfer of each culture at each temperature was then carried through three transfers in pasteurized milk

at 21° C. and the aromas and flavors of these transfers studied. The results obtained were rather uniform with the six butter cultures and are presented in a general way in table III.

TABLE III. AROMAS AND FLAVORS OF BUTTER CULTURES GROWN AT VARIOUS TEMPERATURES

	37° C.	30° C.	25.5° C.	21° C.	19° C.	15° C.
Aroma and flavor after 5 transfers	Decidedly off	Very lacking	Satisfactory	Satisfactory	Slight metallic flavor	Low in acid and flavor
Aroma and flavor after 3 additional transfers at 21° C.	Decidedly off	Very lacking	Satisfactory	Satisfactory	Fair to satisfactory	Fair but rather lacking

The butter cultures carried at 37° C. quickly developed off aromas and flavors and commonly whey was liberated; when transfers were grown at 21° C. the off flavors continued to appear. The cultures carried at 30° C. were very lacking in aroma and flavor and this condition continued in the transfers grown at 21° C. At 25.5° and 21° C. the cultures were regularly satisfactory. The cultures grown at 19° C. had a slight metallic flavor, but transfers carried at 21° C. were fairly satisfactory. The cultures grown at 15° C. were low in acid, aroma and flavor, and transfers grown at 21° C. were somewhat deficient in aroma and flavor.

When the cultures that developed off aromas and flavors as a result of being carried at 37° C. were cultured on beef infusion agar slants, the growth indicated the presence of other than the normal butter culture organisms. The organisms which survive the high pasteurization exposures used in the preparation of milk for butter cultures undoubtedly develop much better at 37° C. than at 21° C., and at the latter temperature they may be completely controlled by the normal butter culture types. The failure of the butter cultures that had been carried at 37° C. to develop a satisfactory aroma and flavor when returned to 21° C. may have been due to a combination of factors. The rapid growth of the contaminating bacteria may have tended to destroy the relationship between the two butter culture types so that a satisfactory balance was not reestablished. Certain of the citric acid fermenters do not develop on agar slopes at 37° C. and may also fail to grow in milk so that they quickly disappeared from the butter cultures.

In a study of the influence of temperature of incubation on the quality of butter cultures, Toens and Hammer⁶ developed satis-

⁶Toens, P., and Hammer, B. W. Studies on starters. Part I. Influence on starters of air supply, temperature of incubation, and rate of ripening. Iowa Agr. Exp. Sta., Res. Bul. 85. 1925.

factory cultures over a rather wide temperature range—at least from 18° to 32° C.—but not at 37° C. These investigators found it impossible to trace a relationship between the favorable effect of a given temperature and certain butter cultures, but they suggested the possibility of a certain temperature being more favorable for one culture than for another.

INFLUENCE OF HOLDING RIPENED CULTURES AT VARIOUS TEMPERATURES

From each of six butter cultures, inoculations were made into five glass stoppered bottles of pasteurized milk in the usual manner. When a culture had developed, one bottle was held at each of the following temperatures: 37°, 30°, 21° and 7° C.; from the fifth bottle, six small bottles of about 25 ml. capacity were filled, the bottles stoppered with corks and sealed with wax and then placed in a cooler at approximately -10° C. Transfers were made daily to pasteurized milk from each bottle held at the four highest temperatures and the quality of the resulting cultures studied. At intervals of one month a small bottle from each series held at -10° C. was thawed, inoculated into pasteurized milk and the quality of the resulting cultures studied.

The results secured with the six butter cultures indicated that at 37° C. ripened butter cultures survived holding for only one day, at 30° C. for only three days, at 21° C. for about seven days, at 7° C. for about one month and at approximately -10° C. for about two months.

INFLUENCE OF ALTERNATE FREEZING AND THAWING

Fifteen butter cultures that had been ripened in pasteurized milk with the usual procedure were put into a cooler at approximately -10° C. Each day they were brought out, allowed to reach a temperature of about 10° C., inoculations made into pasteurized milk and the butter cultures then returned to the cooler. The rate of coagulation and the aroma and flavor of each of the transfers were studied. In general, the cultures withstood freezing and thawing from 5 to 7 times without a change in their activity when inoculated into pasteurized milk; when frozen and thawed from 7 to 10 times there was delayed coagulation in the transfers but the aromas and flavors were quite satisfactory; when frozen and thawed from 10 to 15 times the transfers failed to coagulate.

VI. INFLUENCE OF TEMPERATURE ON THE CONSTITUENT TYPES OF ORGANISMS IN PURE CULTURES

S. LACTIS

(a) IN LITMUS MILK

Each of six *S. lactis* strains which yielded satisfactory butter cultures when combined with suitable citric acid fermenters was

inoculated into nine tubes of litmus milk. After the milk had coagulated a tube from each set was held at the following temperatures: 37°, 30°, 21° and 7° C.; five tubes from each set were placed in a cooler at about -10° C. At various intervals a transfer was made with a loop from each culture at the four highest temperatures to a tube of litmus milk and one of the tubes from each lot held at about -10° C. was thawed and a loop transferred to a tube of litmus milk. The inoculated tubes were held at approximately 21° C. and the rates of coagulation compared with the rates of tubes inoculated with cultures of the same strains that had been transferred daily at 21° C. When a culture that had been held gave about as rapid coagulation as the comparable one that had been transferred daily, each was combined with the same citric acid fermenter, the mixtures were run through five transfers in litmus milk and then inoculated into pasteurized milk: after coagulation, the pasteurized milk cultures were examined for aroma and flavor.

The periods during which the *S. lactis* strains remained in such a condition in litmus milk that they were as satisfactory for the development of butter cultures as cultures transferred daily at 21° C. were as follows: one day at 37° C., three days at 30° C., six days at 21° C., at least three months at 7° C., and least five months at about -10° C.

(b) IN MILK CONTAINING CaCO_3

The influence of temperature on *S. lactis* strains in milk to which 5 percent CaCO_3 had been added was studied, using the six cultures and essentially the same procedures that were employed in studying this type in litmus milk. The CaCO_3 remained in a layer at the bottom of each tube during the growth period but was well distributed through the coagulated milk by shaking just before the tubes were put at the various temperatures and did not again settle out.

The periods during which the *S. lactis* strains remained in such a condition in milk containing CaCO_3 that they were as satisfactory for the development of butter cultures as cultures transferred daily at 21° C. were as follows: four days at 37° C., seven days at 30° C., one month at 21° C., at least five months at 7° C. and at least five months at about -10° C. The presence of CaCO_3 , accordingly, greatly increased the time that a milk culture of a *S. lactis* strain remained in a condition satisfactory for the development of butter cultures.

In every instance when a *S. lactis* strain from either litmus milk or milk containing CaCO_3 coagulated milk reasonably rapidly, it gave satisfactory results in the development of butter cultures; it should be noted that the strains used were originally

selected on the basis of their suitability for the development of butter cultures when combined with certain citric acid fermenters.

CITRIC ACID FERMENTERS

(a) IN LITMUS MILK

Fifteen citric acid fermenters which produced satisfactory butter cultures when combined with a suitable *S. lactis* strain, were each inoculated into two tubes of litmus milk. One tube was sealed with a cork and paraffin while the other was left with an ordinary cotton stopper. At intervals of one month a loop of material from each tube—one sealed and one not—of each citric acid fermenter was transferred to litmus milk, the milk incubated three days at about 21° C. and a loop of the culture thus secured transferred to litmus milk along with a loop of a suitable *S. lactis* strain. As a control, each citric acid fermenter from a culture representing a series of fairly frequent transfers through litmus milk was combined with the *S. lactis* strain. The three combinations with each citric acid fermenter were carried through five transfers in litmus milk and then inoculated into pasteurized milk. The final cultures were examined for aroma and flavor.

The cultures held with cotton stoppers were completely dry in about four months but yielded satisfactory butter cultures until shortly before this time. The sealed cultures gave satisfactory butter cultures for at least eight months.

(b) IN MILK CONTAINING CaCO_3

Eight citric acid fermenters were inoculated into small bottles that had been sterilized after adding 20 ml. of skim milk and 1 gm. of CaCO_3 . The bottles were corked, sealed with wax and the cultures then incubated at about 21° C.; after seven days the bottles were thoroughly shaken to distribute the CaCO_3 . At intervals of three months transfers were made from the bottles, and the young cultures thus secured were compared, from the standpoint of value in the development of butter cultures, with young cultures representing a series of fairly frequent transfers through milk.

The cultures in milk containing CaCO_3 were still satisfactory for the development of butter cultures after one year at room temperature; this commonly approximated 21° C., but during the summer months occasionally reached 37° C.

The studies on the citric acid fermenters indicated that whenever a culture was alive it was in a condition satisfactory for the development of butter cultures; it should be noted that the strains studied were originally selected on the basis of their suitability for the development of butter cultures when combined with certain *S. lactis* strains.

VII. INFLUENCE OF CARRYING BUTTER CULTURES IN DAIRY PLANTS

Butter cultures which had been propagated in dairy plants for varying periods were occasionally brought to the laboratory and run through a series of transfers so that they could be compared with cultures from the same sources that had been carried in the laboratory. A few of these butter cultures were of fine quality, even after having been transferred in dairy plants for more than a year, and had aromas and flavors as desirable as those of the laboratory cultures. Others were less satisfactory and showed coarse, high-acid flavors; since certain *S. lactis* strains give coarse, high-acid flavors the butter cultures may have been contaminated in the plants with organisms of this type.

The plating of butter cultures brought to the laboratory from dairy plants sometimes yielded organisms other than the typical butter culture streptococci; these included bacteria, yeasts and molds. In one series of 10 butter cultures, 6 were found to contain molds; the aromas and flavors of these 6 cultures did not suggest contamination with molds at any time during a number of transfers in the laboratory.

In a study of the keeping qualities of butter cultures that had been held for considerable periods under various temperature conditions, coagulation of the pasteurized milk into which the cultures were inoculated was frequently very slow. The coagulated milk sometimes had a distinct off aroma and flavor of such a character that objectionable types of organisms were evidently involved. Beef infusion agar slope cultures of such materials showed contaminating organisms, and commonly they could also be found microscopically. In general, the organisms were rods; presumably they resisted the pasteurization exposure and, because of the slow acid development, multiplied to a considerable extent. The off odor and flavor of these butter cultures commonly persisted through a series of transfers.

Occasionally butter cultures of a very satisfactory quality have been found to contain contaminating organisms, both by beef infusion agar slope cultures and microscopic examinations, and sometimes these organisms have been present in large numbers. In some instances such organisms have been isolated and their influence on the aroma and flavor of a butter culture studied by inoculating two bottles of pasteurized milk with a satisfactory butter culture and adding the organism to one of the bottles. The results showed that certain organisms have no influence on the aroma and flavor of a butter culture, even when they are present in large numbers. One type of contaminating organism that was occasionally encountered could be eliminated from a butter culture by allowing the ripened cul-

ture to stand at 21° C. for about two days; presumably the contaminating type could not resist continued exposure to considerable acid.

VIII. EFFECT OF THE GERMICIDAL PROPERTY OF MILK ON BUTTER CULTURES

Under dairy plant conditions milk is occasionally encountered which, following inoculation with a vigorous butter culture, coagulates slowly. Commonly such milk has been heated to comparatively low pasteurization temperatures. Hammer and Baker⁷ found that butter cultures developed acid more slowly in milk heated to 62.8° C. for 30 minutes than in milk heated to considerably higher temperatures for the same time; essentially the same rate of development of a butter culture occurred in milk heated to 71.1° C. for 30 minutes as when a higher temperature was used for this period. Under certain conditions, high pasteurization exposures are definitely objectionable—for example, in the making of cottage cheese high temperature pasteurization of milk gives a body that is discriminated against by certain consumers—and, accordingly, high pasteurization exposures are not always a practical remedy for delayed coagulation.

In order to determine whether or not butter cultures are affected differently by the germicidal properties of various lots of milk, eight cultures were studied. A lot of raw milk was distributed to small glass stoppered bottles in 100 ml. quantities and eight bottles pasteurized for 30 minutes at each of the following temperatures: 62.5°, 68°, 71° and 82° C. After cooling the milk, each culture was inoculated uniformly into a series of bottles which represented the various pasteurization exposures and also into unpasteurized milk. The acidity of each bottle was determined frequently by titrating with N/10 NaOH, using phenolphthalein as an indicator.

Two of the butter cultures developed acid and coagulated the milk at about the same rate, whether it was raw or pasteurized with a low or a high exposure. With six cultures acid development and coagulation were delayed in the raw milk and in the milk heated to 62.5° or 68° C.; two of these cultures failed to coagulate the raw milk even after very long holding periods. All eight of the cultures gave the same rate of acid development and coagulation in the milk heated to 71° C. as in that heated to 82° C.

A second trial with the same eight butter cultures gave essentially identical results.

The results indicated that butter cultures vary a great deal in their resistance to the germicidal property of milk. There

⁷Hammer, B. W., and Baker, M. P. Studies on starters. Iowa Agr. Exp. Sta., Res. Bul. 106. 1928.

is, moreover, no assurance that a culture which is resistant in the case of one supply of milk will be resistant with another, and on the basis of the studies that have been reported for various organisms, differences would be expected. The data suggest that if milk from a given supply is coagulating very slowly with a certain butter culture it may be possible to develop a culture that will not be influenced in this way.

CONCLUSIONS

1. *S. lactis* var. *maltigenes*, *S. lactis* var. *hollandicus*, *S. lactis* var. *tardus* and *S. thermophilus* have characters which make them unsatisfactory for the development of butter cultures.

Combinations of various strains of *S. lactis* var. *maltigenes* and citric acid fermenting streptococci developed a malty odor and flavor through a series of transfers.

2. Various strains of *S. lactis* var. *anoxyphilus* in combinations with citric acid fermenting streptococci sometimes yielded fairly satisfactory butter cultures.

3. Typical *S. lactis* strains which produced sharp, high-acid flavors when grown alone generally gave this same condition in combinations with citric acid fermenters.

4. Typical *S. lactis* strains showing a flavor suggesting cabbage when grown alone produced this same condition in combinations with certain citric acid fermenters, while with others satisfactory butter cultures were secured.

5. Typical *S. lactis* strains showing no objectionable flavors when grown alone gave satisfactory butter cultures with certain citric acid fermenters, while with others they did not.

6. With unselected *S. lactis* and citric acid fermenting strains, a *S. lactis* strain that combined satisfactorily with one citric acid fermenter often did not combine satisfactorily with another, and a citric acid fermenter that combined satisfactorily with one *S. lactis* strain often did not combine satisfactorily with another.

7. The volatile acidities produced in milk by 71 citric acid fermenting streptococci varied from 7.1 to 30.0 and averaged 20.3, while in milk plus 0.4 percent citric acid the volatile acidities ranged from 25.0 to 84.8 and averaged 57.2.

8. There was no correlation between the amount of volatile acid formed and the ability of a citric acid fermenter to produce a satisfactory butter culture in combination with *S. lactis* A, a strain which was very satisfactory for the development of butter cultures.

9. The citric acid fermenters that improved the flavor of *S. lactis* strains produced the most improvement with *S. lactis* strains that had a clean, mild flavor when grown alone.

10. A citric acid fermenter which gave an odor suggesting a

butter culture in milk did not always develop a good butter culture when combined with a *S. lactis* strain producing a clean, mild flavor.

11. There was no correlation between the rate of volatile acid production of a citric acid fermenter in pure culture and its suitability for the development of butter cultures.

12. A certain combination of a citric acid fermenter and a *S. lactis* strain was very constant in its ability or inability to develop a satisfactory butter culture.

13. Some combinations of organisms produced a butter culture aroma and flavor in the first transfer in pasteurized milk, while with others several transfers were lacking in aroma and flavor and the desired aroma and flavor then appeared. Some combinations produced a butter culture aroma and flavor in the first few transfers while the later transfers were deficient.

14. A *S. lactis* strain selected on the basis of its ability to produce a satisfactory butter culture when combined with the citric acid fermenter of a certain combination also combined satisfactorily with the citric acid fermenters that produced satisfactory butter cultures in mixtures with the *S. lactis* of the combination; a citric acid fermenter selected on the basis of its ability to produce a satisfactory butter culture when combined with the *S. lactis* of a certain combination also combined satisfactorily with the *S. lactis* strains that produced satisfactory butter cultures in mixtures with the citric acid fermenter of the combination.

15. Rapid procedures for the selection of organisms satisfactory for the development of butter cultures were advantageous since comparatively few combinations of identified organisms were satisfactory.

16. Although all of the transfers of a butter culture carried under rather careful conditions were not equally good, with reasonably uniform methods of propagation butter cultures showed a surprising stability.

17. Regardless of the comparative numbers of the two types of organisms in the original mixture, combinations of *S. lactis* and a citric acid fermenter established the same relationship so that the same general aroma and flavor were developed.

18. The addition of a citric acid fermenter at the time a butter culture which was lacking in aroma and flavor was inoculated into pasteurized milk regularly failed to improve the butter culture.

19. Butter cultures were carried satisfactorily by making transfers every other day in tubes of sterile litmus milk and incubating at about 21° C.; the overripening which followed the long period of incubation was not harmful.

20. The two constituent organisms of butter cultures tend-

ed to maintain a relationship that was satisfactory for the production of a desirable aroma and flavor, even when the milk in which they grew was not coagulated during a series of transfers, but it was possible with a long series of rapid transfers to destroy the desired relationship between the types.

21. Butter cultures developed better at 25.5° or 21° C. than at 37°, 19° or 15° C.; 37° C. favored the growth of organisms surviving the high pasteurization exposures, while 19° and 15° C. greatly decreased the rate of coagulation of the butter cultures.

22. The maximum periods ripened butter cultures could be held at various temperatures and still yield satisfactory butter cultures when transferred were as follows: one day at 37° C., three days at 30° C., about seven days at 21° C., about one month at 7° C., and about two months at approximately -10° C.

23. In general, butter cultures withstood freezing and thawing from 5 to 7 times without destroying their ability to produce satisfactory butter cultures; when frozen and thawed from 7 to 10 times there was delayed coagulation on transferring, but the aromas and flavors were quite satisfactory; when frozen and thawed from 10 to 15 times the cultures failed to coagulate milk into which they were inoculated.

24. The maximum periods *S. lactis* cultures in litmus milk could be held at various temperatures and remain as satisfactory for the development of butter cultures as cultures regularly transferred at 21° C. were as follows: one day at 37° C., three days at 30° C., six days at 21° C., at least three months at 7° C. and at least five months at about -10° C. The addition of CaCO_3 to the milk greatly increased the time that a *S. lactis* strain remained in a condition satisfactory for the development of butter cultures.

25. Litmus milk cultures of citric acid fermenting streptococci remained satisfactory for the development of butter cultures at room temperature for at least eight months in sealed test tubes and for about four months, or until shortly before the cultures were completely dry, in test tubes with which evaporation was not checked. When CaCO_3 was added to the milk, the cultures were satisfactory for the development of butter cultures after one year at room temperature.

26. The citric acid fermenters remaining alive after being held at room temperature, either in litmus milk or in milk plus CaCO_3 , for long periods were regularly satisfactory for the development of butter cultures.

27. Butter cultures propagated in dairy plants for varying periods were sometimes as satisfactory as the cultures sent to the plants, but in other instances they had been contaminated.

28. Some butter cultures were greatly affected by the germicidal properties of milk while others were not.

